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Ontogeny of Osmoregulatory Structures in the Shrimp *Penaeus japonicus* (Crustacea, Decapoda)

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Abstract. The ontogeny of differentiated osmoregulatory epithelia in the branchial chamber (gills, branchiostegite, pleura, epipodite) was studied by transmission electron microscopy throughout the postembryonic development of *Penaeus japonicus*. These epithelia are characterized by typical cytological features, including apical microvilli and numerous basal infoldings associated with mitochondria.

Differentiated osmoregulatory structures are not observed in the early larval stages: nauplii and zoea 1. In the next larval stages, zoeas and mysis, gills and epipodites are developed as buds only, but osmoregulatory epithelia are observed in the branchiostegites and pleurae. The differentiated structures of the branchiostegites and pleurae are still present in postlarvae but disappear in juveniles and adults. Gills and epipodites develop progressively in the postlarval stages, with early differentiation of osmoregulatory epithelia in the epipodites. In juveniles and adults, the gill epithelium is poorly differentiated; in contrast, abundant differentiated osmoregulatory structures are observed in the epipodites.

Ontogenetical comparisons of these observations with previous studies in the same species reveal strong correlations between the development of osmoregulatory epithelia, the ability to osmoregulate, the activity of Na^+-K^+ ATPase, and salinity tolerance during the postembryonic development of *Penaeus japonicus*.

Introduction

Among the few comprehensive histological studies of osmoregulatory structures in decapod crustaceans, most have concerned the gills of crabs (Drach, 1930; Chen, 1933; Smyth, 1942) and of shrimps such as *Palaemonetes varians* (Allen, 1892), *Crangon vulgaris* (Debaisieux, 1970), and *Penaeus aztecus* (Foster and Howse, 1978).

Ultrastructural studies of the gills of adult crustaceans are numerous (review in Bouaricha, 1990), but to our knowledge, comparable information about larvae and postlarvae is lacking. In penaeid shrimp, a description of the ontogeny of gills in *Penaeus japonicus* was given by Hudinaga (1942), but without histological data.

Gills are among the most permeable external surfaces of crustaceans, and they are considered the primary site for ionic and osmotic regulation (Robertson, 1960; Lockwood, 1962, 1968; Gilles, 1975; Croghan, 1976; Kirschner, 1979; Pequeux and Gilles, 1981, 1988; Towle, 1984a). In some earlier studies, patches of salt-transporting tissue were also described on the branchial chamber epithelium in larvae of *Penaeus aztecus* (Talbot *et al.*, 1972) and *Callianassa jamaicense* (Felder *et al.*, 1986).

This study presents a general and histological description of the gills in juvenile and adult *P. japonicus* and their ontogeny during larval and postlarval development, with particular attention directed at the localization of epithelia involved in osmoregulation. We also looked for similar tissues in the branchiostegite, pleura, and epipodite. Our ultimate goal was to evaluate the validity of a hypothetical relationship between the ontogeny of osmoregulatory structures in gills and other epithelia and the ontogeny of osmoregulation demonstrated by the changes in osmoregulatory capacity (Charmantier, 1986; Charmantier *et al.*, 1987, 1988; Charmantier-Daures *et al.*, 1988) and in the activity of Na^+-K^+ ATPase (Bouaricha, 1990; Bouaricha *et al.*, 1991) during the postembryonic development of *P. japonicus*.

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Material and Methods

Animals

Penaeus japonicus larvae, postlarvae, and adults were obtained from the Ifremer center (Deva-Sud, Palavas, Hérault, France) and from a local shrimp-farm (Poissons du soleil, Balarue les Bains, Hérault). The different developmental stages were identified according to morphological criteria (Hudinaga, 1942). Larval development consists of six naupliar, three zoea, and three mysis stages. A metamorphic molt transforms the late mysis 3 larva into the first postlarval stage. Postlarvae progressively acquire the adult morphology through about 20 molts. Postlarval stages are designated by an abbreviation of stage; *e.g.*, PL4 for fourth stage postlarva. Molt stages were controlled by microscopical observation of pleopods according to the technique widely used with adult crustaceans (Drach and Tchernigovtzeff, 1967); only animals in stage C were used.

Microscopy

All stages were fixed in Halmi's fluid, sectioned in the transverse plane, stained with Masson's trichrome (variant Goldner; Martoja and Martoja, 1967), and examined with a light microscope. For electron microscopy, animals were fixed in 2.5% glutaraldehyde in 0.1 *M* saline cacodylate buffer adjusted to the osmotic pressure of seawater with sodium chloride. Samples were post-fixed in 1% OsO_4 in the same buffer and embedded in Spurr's medium. Ultrathin sections, stained sequentially with uranyl acetate and lead citrate, were examined with a JEOL 200CX electron microscope at 100 kV.

Results

Structure of gills in juvenile and adult

Eighteen gills are present in each branchial chamber: one podobranchial gill, eleven arthrobranchial gills, and six pleurobranchial gills. In *P. japonicus*, as in other species of penaeid shrimps, all gills are dendrobranchiate, and this consists of an axis that supports a series of paired branches set at right angles along its length (Fig. 1). Each branch then gives rise to perpendicularly oriented filaments that bifurcate at least once (Figs. 2 and 3). Mucus pores are located on both sides of the axis.

The internal structure of the gills includes a longitudinal septum dividing the lumen of each axis, branch, and filament into afferent (external) and efferent (internal) vessels (Figs. 3 and 4).

The histology of the gill is as follows. The surface of each branch or filament is covered by a thin cuticle that overlies a simple epithelium. A central lacunar system expands in the tip of each filament into a hemolymphatic lacuna. Connective tissues are present in the septa of filaments and axis of the gill (Fig. 4).

Changes in gill structure during development

In mysis stages 2 and 3, the branchial chamber is widely open. The gills, appearing as small buds on the coxopodite of the thoracic appendages, are limited by a simple epithelium enclosing a hemolymph lacuna (Fig. 5).

In postlarvae PL1, the curved larger branchiostegite partly encloses the branchial chamber. The gill bud is longer, and the hemolymph lacuna is wider (Fig. 6).

In PL4, the septum that divides the axis of the gill is present. The gill epithelium gives rise to branches (Fig. 7) that are present along the whole length of the gill in PL5. At this stage, afferent and efferent vessels are differentiated in the largest gills, and the branchial chamber is completely enclosed by a lateral-ventral infolding of the branchiostegite. In PL10, the gills are about 1 mm long. The epithelium on the surface of the branches is only 0.1–0.7 μm thick, except near the nuclei; few organelles are visible in the cytoplasm (Fig. 8). Only this type of slightly differentiated epithelium was observed on transverse and longitudinal serial sections of the gills.

In 3-month-old juveniles, the epithelium of the branches and filaments (Figs. 9 and 10) is about 0.8 μm thick; it is thicker near nuclei (1.4–2.2 μm) and at the tip of the filaments (1.5 μm) that are widened to form a distal lacuna. The cytoplasm contains but few organelles (Fig. 10), and these include spherical (0.4 μm) or elongated (1 μm) mitochondria. Microvilli occur infrequently on the apical cytoplasmic membrane. Infoldings of the basal lamina are limited (Fig. 9).

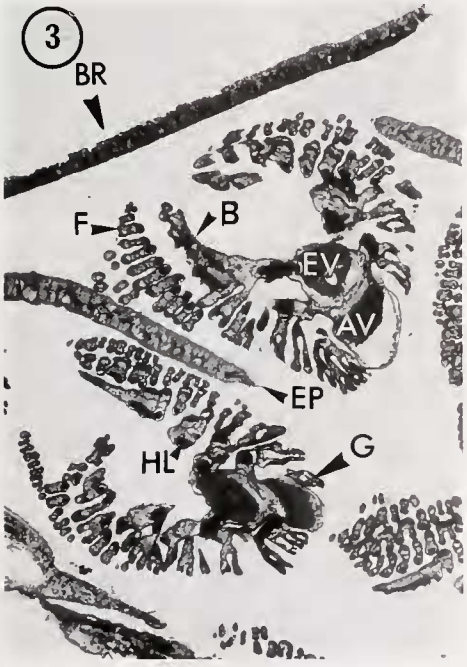
In 5-month-old juveniles, the apical cytoplasmic membrane of the gill epithelium is differentiated, giving rise to microvilli, 0.3–0.8 μm high, that delimit important subcuticular spaces (Fig. 11). The cytoplasm contains many large mitochondria, but other organelles are still scarce.

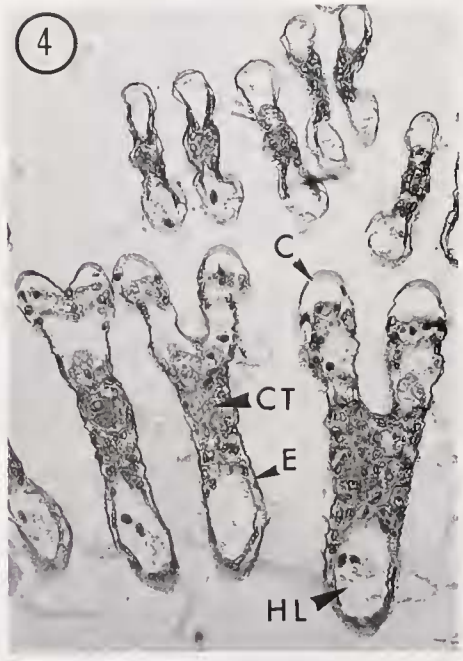
No difference in structure was noted between the different filaments along the length of the gill axis, or between the anterior and posterior gills.

Pleurae

The pleural epithelium covers the lateral internal surface of the branchial chamber (Figs. 5 and 24).

In zoea 2 larvae, this epithelium presents two aspects: thin (1.4–4 μm) and only slightly differentiated, or thicker (4–7 μm) and with typical differentiations, *i.e.*, large invaginations of the basal membrane enfolding mitochondria (Fig. 13). The apical cytoplasm contains the nucleus, numerous vesicles of reticulum, free ribosomes, and mitochondria (Fig. 12).



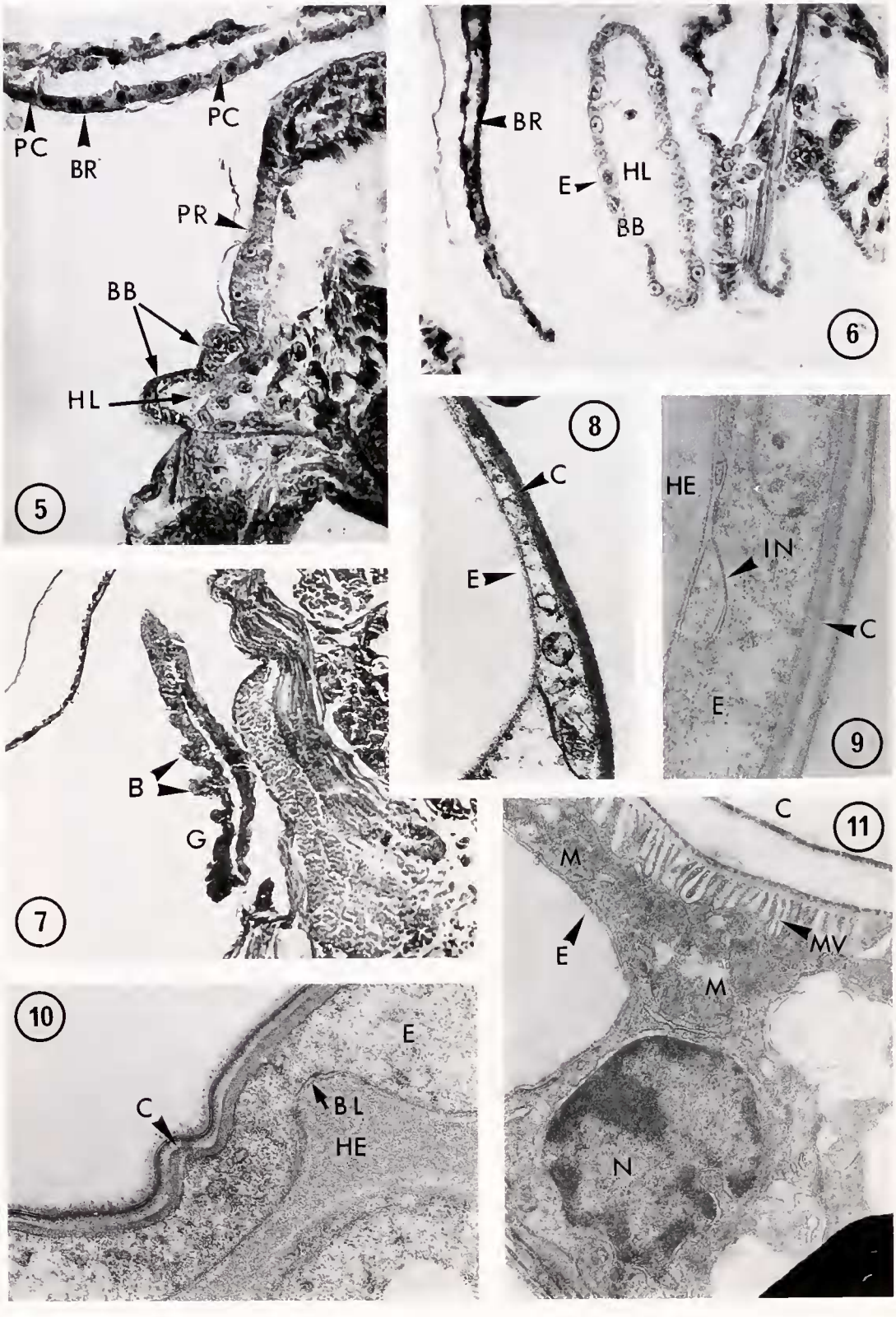


Figures 1–4. Gills of juvenile *Penaeus japonicus*. A: axis, AV: afferent vessel, B: branches, BR: branchiostegite, C: cuticle, CT: connective tissue, E: epithelium, EP: epipodite, EV: efferent vessel, F: filament, G: gills, HL: hemolymphatic lacuna.

Figures 1–2. Dorsal face of a dendrobranchiate gill showing the axis and the filaments (×25 and ×90, respectively).

Figure 3. General organization of the branchial chamber of a juvenile (×250). The outside of the branchial chamber is closed by the branchiostegite. Subtransverse sections of gills show the axis divided into afferent and efferent vessels and branches and filaments. Epipodites are intercalated between the gills.

Figure 4. Transverse section of gill filaments in an old juvenile. The monolayer epithelium is covered by a thin cuticle. Each tip of the filament is occupied by a hemolymphatic lacuna.



Figures 5–11. Ontogeny and structure of gills in *Penaeus japonicus* B: branches, BB: branchial bud, BL: basal lamina, BR: branchiostegite, C: cuticule, E: epithelium, G: gill, HE: hemolymph, HL: hemolymphatic lacuna, IN: infolding, M: mitochondria, MV: microvilli, N: nucleus, PC: pillar cell, PR: pleura.

Figure 5. Mysis 3 larva (× 400). Early gill buds on a thoracic appendage.

In mysis 3 larvae, the two forms of pleural epithelium are also present. The differentiated epithelium is located in the central region of the pleural dorsoventral axis. Differentiations are more pronounced than in zoeas (Fig. 14). Although the total thickness of the epithelium is similar (4–8 μm) to the previous stage, basal infoldings have expanded (1.2–3 μm); mitochondria are very abundant; and the apical membrane bears thin microvilli, 0.8 μm long, either scarce or close-ranked.

In postlarvae, although observations were available from light microscopy only, the differentiations present in mysis seem to have been retained. Longitudinal streaks that characterize basal infoldings can be observed on epithelial cells of the pleura.

In adults, no differentiated epithelial structure was observed on any of several serial transverse sections of the pleura.

Branchiostegites

The branchiostegite is almost horizontally oriented in the larval stages, resulting in an open branchial chamber. In later stages, the chamber is progressively closed by the folding down of the branchiostegite (Figs. 5 and 18). At all the developmental stages, the branchiostegite comprises two simple epithelia, external and internal, separated by a hemolymph lacuna. The epithelia are linked by pillar cells (Fig. 5). The external epithelium, under the cuticle, is thin and slightly differentiated. The internal epithelium is thicker, and its structure varies with the developmental stage.

In stage mysis 3, the internal epithelium is 8 μm thick and shows differentiated zones similar to those described for the pleura (Fig. 15); the apical cytoplasmic membrane presents patches of microvilli, 0.1–1 μm long. The basal cytoplasmic membrane forms many deep infoldings separating cytoplasmic areas that contain abundant round or elongated mitochondria. Nuclei are located in the narrow median zone, above the basal infoldings. They are large and oval (2.6 × 4 μm), with spots of chromatin; the external layer of the nuclear membrane carries numerous ribosomes. Rough endoplasmic reticulum and irregularly shaped mitochondria can be observed around the nuclei.

In PL 10 postlarvae, the internal epithelium is thicker (12–14 μm) and presents the same differentiations as in the mysis (Figs. 16 and 17), with long (1–2 μm) and abundant microvilli at the apical pole (Fig. 16) and with numerous elongated basal infoldings, sometimes nearly as high as the cell. Many elongated mitochondria are located between the infoldings and under the microvilli.

In adults, this type of differentiated epithelium was not observed in any of the serial sections of the branchiostegite. The internal epithelium is composed of high cells. The nucleus is centrally located. The cytoplasmic membrane shows ample, sinuous interdigitations. Different organelles can be observed in the cytoplasm: abundant rough endoplasmic reticulum forming a tubular network connected to small vesicles, elongated mitochondria, large golgi profiles with numerous vesicles, and oriented fibril bundles. Apical microvilli or basal infoldings were not observed.

Epipodite

The epipodites, or mastigobranchs, are elongated, thin, biramous structures, attached to coxopodites of some thoracic appendages. Six of them are present in each branchial chamber of adult shrimp.

In mysis 2 and 3 larvae, the first epipodites appear as small buds. This aspect is retained until the early postlarval stages. They lengthen and become biramous and covered with setae in PL5. According to Hudinaga (1942), their full complement is reached by PL11.

In PL5 postlarvae, epipodites are formed of a simple epithelium enclosing a vascular network locally enlarged in lacunae (Figs. 18 and 19); the tip is widened around a hemolymph lacuna. In light microscopy, the basal part of the cells presents a striated appearance, similar to the differentiated zones observed in the branchiostegite and the pleurae.

In 5-month-old juveniles and in adults, epipodites present two simple epithelia separated by a central connective tissue. The epithelium is made of high columnar cells (30 μm in adults), with the nuclei located in the apical part of the cell under a thin cuticle (Fig. 20). The ultrastructure of the epithelial cells features both apical and basal infoldings (Figs. 21, 22, and 23). The apical infoldings are oriented at right angles to the cuticle. Mitochondria, tubules of rough endoplasmic reticulum, and numerous small round vesicles are located between them (Fig. 21). The basal infoldings are organized as a compact network, penetrating deeply, up to 20 μm, into the cytoplasm. Abundant elongated mitochondria (0.4 × 2.5 μm) are inserted between the infoldings.

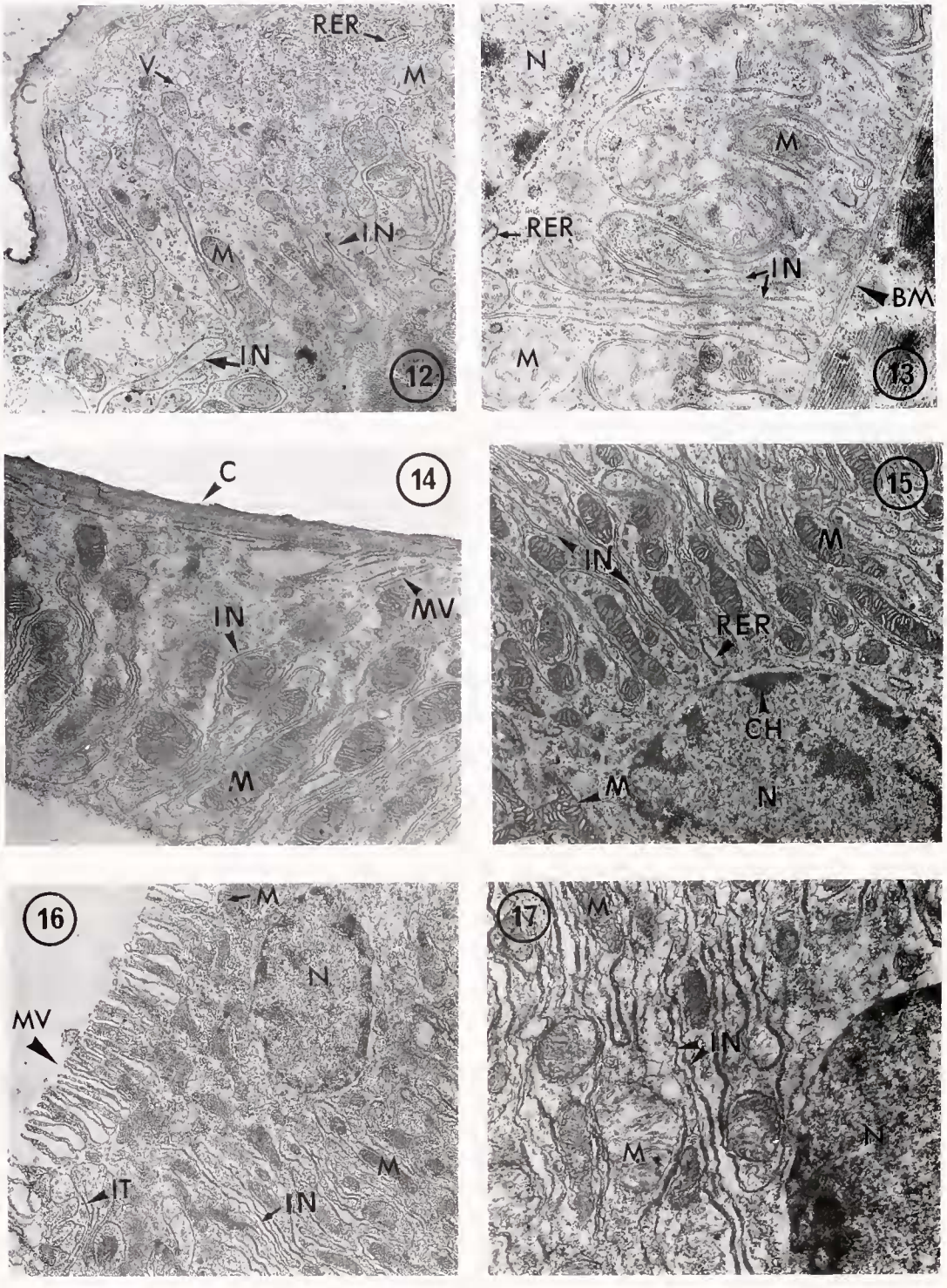
Figure 6. Postlarva PL 1 (×400). Gill bud structure: a thin epithelium delineating a central hemolymphatic lacuna.

Figure 7. Postlarvae PL 4 (×400). First branchial branches on a gill.

Figure 8. Postlarvae PL 10 (×13000). Epithelium of a branchial branch.

Figures 9–10. Juvenile (×17500). Branchial epithelium. Thin epithelium and few intracellular organelles.

Figure 11. Juvenile (×8000). Branchial epithelium with apical microvilli and some mitochondria.



Figures 12–17. Pleura and branchiostegite of larvae and postlarvae of *Penaeus japonicus*. BM: basal membrane, C: cuticle, CH: chromatin, IN: infoldings, IT: interdigitation, M: mitochondria, MV: microvilli, N: nucleus, RER: rough endoplasmic reticulum, V: vesicle.

Figures 12–13. Pleura, zoea 2 (×9200 and ×15000, respectively). Pleural epithelium with numerous basal infoldings associated with mitochondria.

Figure 14. Pleura, mysis 3 (×9100). The pleural epithelium contains two types of differentiations: apical microvilli and basal infoldings with mitochondria.

Figure 15. Internal layer of the branchiostegite, mysis 3 (×8300). Numerous basolateral infoldings associated with mitochondria.

Discussion and Conclusions

Gills

Gill buds appear at the end of the larval phase, and their development progresses during the postlarval period. The branchial formula is complete at stage PL 11 (Hudinaga, 1942).

Only one type of gill epithelium was observed in the postlarvae. This thin epithelium containing few nuclei and very few organelles was not differentiated. A similar type of epithelium was described in adult *Callinectes sapidus* (Copeland and Fitzjarrell, 1968) and *Ocypode ceratophthalma* (Storch and Welsch, 1975). These observations, and our own, suggest that this epithelium is probably involved in gas exchange.

The epithelium begins to differentiate in juveniles with the formation of microvilli on the apical cytoplasmic membrane of the cell, under the cuticle. The differentiation progresses in juveniles that are more than 5 months old, resulting in a higher density of mitochondria under the microvilli.

In juveniles and adults, we found that the epithelium of the gills is formed of thin cells having, as their only apical differentiated structures, microvilli and mitochondria. These cells were also described in *Penaeus aztecus* (Foster and Howse, 1978) and in *Palaemontes pugio* (Doughtie and Rao, 1978). They are the principal cells of the gills of palaemonid and penaeid shrimps (Taylor and Taylor, 1992). Similar epithelia have been reported in the posterior gills of *Eriocheir sinensis* (*Barra et al.*, 1983) and *Gecarcinus cruentata* (Martelo and Zanders, 1986). This type of epithelium could be involved in osmoregulation, a hypothesis supported by the increase in the size of the microvilli in *P. aztecus* that were transferred to low or high salinities (0.9 and 59‰; Foster and Howse, 1978).

The comparison with other species demonstrates that the gill epithelium is only slightly differentiated in *P. japonicus*. In *Gecarcinus lateralis* (Copeland, 1968), *Callinectes sapidus* (Copeland and Fitzjarrell, 1968), *Astacus leptodactylus* (Bielawski, 1971), *Astacus pallipes* (Fisher, 1972), *Gecarcinus oceanicus* (Milne and Ellis, 1973), *Procambarus clarkii* (Burggren *et al.*, 1974), *Jaera nordmanni* (Bubel, 1976; Bubel and Jones, 1974), *Asellus aquaticus* (Babula, 1979), *Holthuisana transversa* (Taylor and Greenaway, 1979), *Eriocheir sinensis* (Barra *et al.*, 1983), *Uca mordax* (Finol and Croghan, 1983), and *Crangon crangon* (Papathanassiou, 1985), the gill epithelium is characterized by apical microvilli and basolateral infoldings associated with mitochondria. These ultrastructural features are typical of tissues involved in active ionic transport (Pease, 1956; Berridge and Oschman, 1972). In some of these species—*Gecarcinus lateralis* (Copeland and Fitzjarrel, 1968), *Carcinus maenas* (Goodman and Cavey, 1988, 1990; Compere *et al.*, 1989), *Procambarus clarkii* (Burggren *et al.*, 1974)—two types of epithelia coexist in the gill, a thin one supposedly implicated in gas exchange, and a differentiated epithelium involved in hydromineral exchanges. In adult *P. japonicus*, we observed only one type of epithelium with apical microvilli but without basal infoldings.

In addition, we saw no morphological or ultrastructural differences between the anterior, median, and posterior gills of *P. japonicus*. We thus conclude that a single type of epithelium exists in *P. japonicus*, as in *P. aztecus* (Foster and Howse, 1978) and in the crab *Holthuisana transversa* (Taylor and Greenaway, 1979). In other species, anterior and posterior gills are histologically different. In *Eriocheir sinensis* (Barra *et al.*, 1983), the anterior gills have a thin, little-differentiated epithelium, probably involved in respiration; the epithelium of the posterior gills is differentiated and certainly implicated in osmoregulation. This hypothesis is confirmed by physiological data, since only the posterior gills are involved in osmotic and ionic regulation (Schoffeniels and Gilles, 1970; Pequeux and Gilles, 1981, 1988). This topographic difference has also been observed in *Astacus pallipes* and *A. leptodactylus* (Dunel-Erb *et al.*, 1982) and in *Asellus aquaticus* (Babula, 1979).

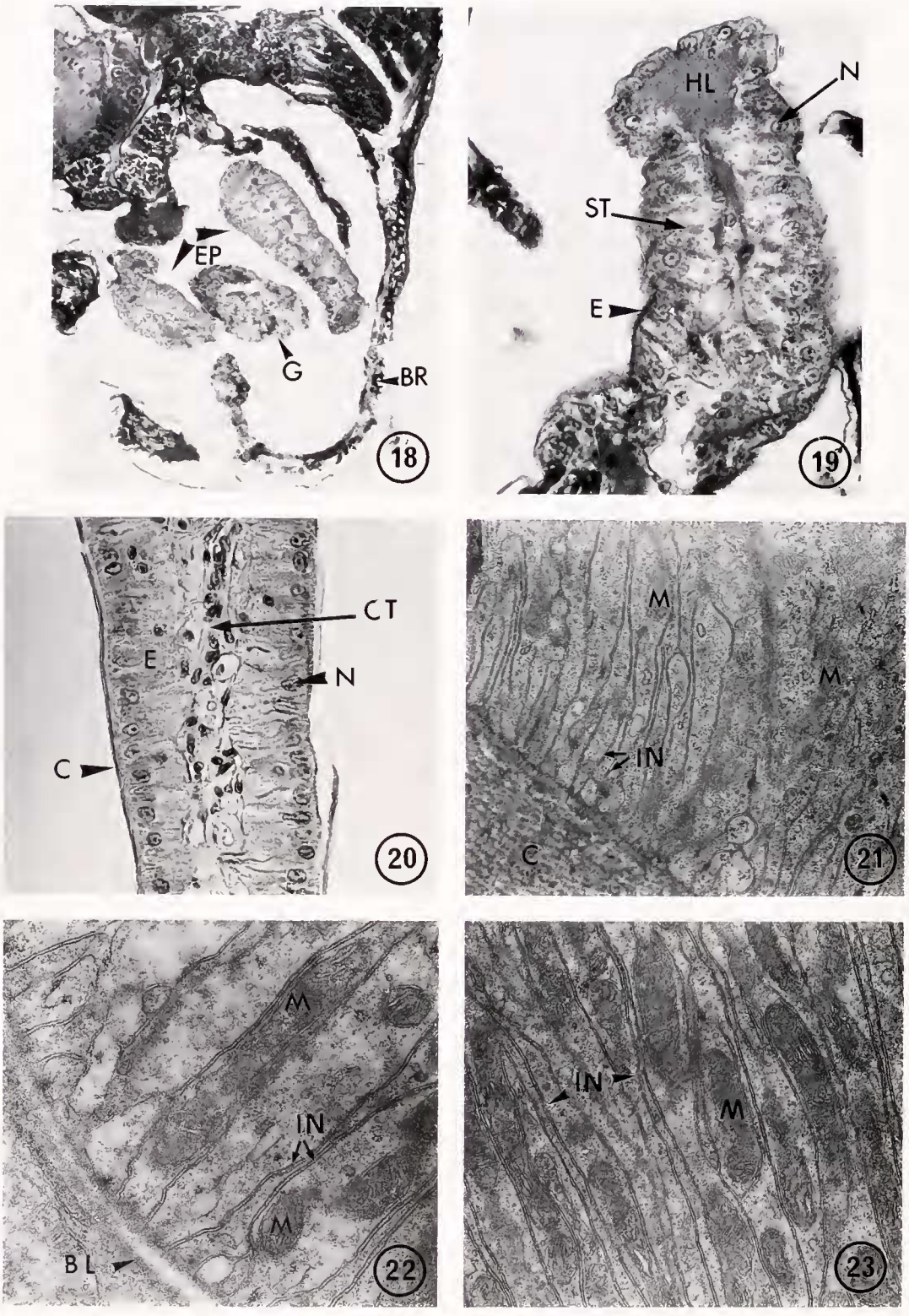
In summary, the gills of *P. japonicus* appear as buds during the last larval stages and grow progressively during the first postlarval stages. The gill epithelium differentiates slightly and progressively in juveniles and adults, with apical microvilli and mitochondria beneath it. The differentiated gills are implicated mainly in respiration and, less than in other species, probably in osmoregulation. This last function is also shared by other structures of the branchial chamber. The structure of the gill epithelium in *P. japonicus* (and probably other penaeids) is simpler than that in most other crustacean groups.

Branchiostegite and pleura

The branchiostegite and pleural epithelia are not differentiated in nauplii and zoea 1. Differentiation appears in zoea 2 and persists in the postlarvae; the most typical features are apical microvilli, deep basal and lateral infoldings, and abundant mitochondria. These elements were called "mitochondrial pumps" by Copeland (1964).

Figure 16. Internal layer of the branchiostegite, postlarvae PL 10 (×6500). Numerous apical microvilli and basal infoldings with mitochondria.

Figure 17. Internal layer of the branchiostegite, postlarvae PL 10 (×13000). Detail of the interrelations between infoldings and mitochondria.



Figures 18–23. Epipodites in postlarvae and adult of *Penaeus japonicus*. BL: basal lamina, BR: branchiostegite, C: cuticle, CT: central connective tissue, E: epithelium, EP: epipodite, G: gill, HL: hemolymphatic lacuna, IN: infoldings, M: mitochondria, N: nucleus, ST: striation.

Figure 18. Transverse section of the branchial chamber, postlarvae PL 5 (×200). The branchial chamber is almost completely enclosed by the folded branchiostegite. Epipodites are intercalated between the gills.

A similar type of epithelium, localized on the internal surface of the branchial chamber, has been found in larvae and postlarvae of *P. aztecus* (Talbot *et al.*, 1972) and in the two zoeal stages of *Callianassa jamaicense* (Felder *et al.*, 1986).

Our observations demonstrate that the differentiation of the branchiostegite and pleural epithelia has disappeared in adults. We hypothesize that branchiostegite and pleural epithelia have an osmoregulatory function in the young stages of *P. japonicus* before the adult structures (*i.e.*, gills and epipodites; see next paragraph) differentiate and take up this function. This hypothesis is supported by the very low level of Na^+-K^+ ATPase activity in the branchiostegite of adult *P. japonicus* (Bouaricha, 1990; Bouaricha *et al.*, 1991). Similar results have been described in *Artemia salina:* the nauplii, which are strong regulators, possess a dorsal gland with the typical differentiations of a tissue implicated in osmoregulation (Conte *et al.*, 1972; Hootman and Conte, 1975). This gland degenerates when "gills" develop (Dejdar, 1930).

Rough endoplasmic reticulum, ribosomes, and golgi profiles are very abundant in the branchiostegite of adult *P. japonicus*, after the regression of its microvilli; this tissue could thus shift from osmoregulation to other functions.

Epipodite

Epipodites and gills appear concurrently during postlarval development. Epipodite cells appear striated (light microscopy) in postlarvae, and electron microscopical observations demonstrate that this epithelium is thicker and more differentiated than that in the other adult tissues studied (*i.e.*, gills, branchiostegites, pleurae). The cells of the epithelium are very similar to the thick cells, or ionocytes, described on the posterior pair of gills in crabs (review in Taylor and Taylor, 1992). Copeland's "mitochondrial pumps" are present in all cells and along the whole length of the epipodites. Very few comparable studies are available in other species. Buchanan (1889) proposed a gill-cleaning function for *Penaeus* epipodites. According to Hudinaga (1942), who described them and included them in the gill formula under the name of mastigobranchia, epipodites are gill structures. In *Crangon vulgaris* (Debaisieux, 1970), the epipodite, as studied by light microscopy, has a thick epithelium. In *Astacus pallipes* and *A. leptodactylus,* Bock (1925) described the epipodites as wide vascular structures offering a large exchange surface; an electron microscopical study of these structures revealed important differentiations similar to those described in *P. japonicus* (Dunel-Erb *et al.*, 1982).

Epipodites and gills have a common phylogenetic origin (Mill, 1972). In decapods, podobranchiate gills originate from epipodites. In *P. japonicus,* podobranchiate gills and epipodites are attached at the same location on the coxopodite of maxillipeds. Their common origin, their topographical and histological similarities, and their features typical of ion-transporting tissues suggest that epipodites are probably involved in osmoregulatory mechanisms. This is further supported by the high level of Na^+-K^+ ATPase activity measured in epipodites of adult *P. japonicus* (Bouaricha, 1990; Bouaricha *et al.*, 1991).

In summary, osmoregulatory structures are progressively established during the postembryonic development of *P. japonicus* (Fig. 24). In larval stages, before gills and epipodites are present, the features typical of osmoregulatory epithelia were observed in pleurae and branchiostegites. During the postlarval stages, the osmoregulatory structures are still present in the branchiostegites and pleurae, but these disappear in the juveniles and adults. Gills and epipodites continue developing during the postlarval stages. In juveniles and adults, the gill epithelium is thin, slightly differentiated, and certainly most involved in respiration. From our observations in adults, both the gills and epipodites could be involved in osmoregulation. But the robust and dense osmoregulatory structures in the epipodites of adult *P. japonicus* point to a major osmoregulatory function of the epipodites, a new finding in penaeid shrimps and in decapod crustaceans.

Relation between the ontogeny of osmoregulatory structures and the ontogeny of osmoregulation

Previous studies conducted in *P. japonicus* on ATPase activity (Bouaricha, 1990; Bouaricha *et al.*, 1991), osmoregulation (Charmantier, 1986; Charmantier *et al.*, 1988), and salinity tolerance (Charmantier *et al.*, 1987; Charmantier-Daures *et al.*, 1988) during the postembryonic development, as well as the results of this study, support the existence of correlations between the establishment of osmoregulatory structures and the ontogeny of osmoregulatory physiological processes.

Figure 19. Epipodite, postlarvae PL 5 (×500). The simple epithelium encloses a vascular network enlarged in a lacuna at the tip.

Figure 20. Epipodite, adult structure. Two simple columnar epithelia, separated by a central connective tissue.

Figures 21–22. Epipodite, adult (×13000 and ×23000). Apical (Fig. 21) and basal (Fig. 22) part of the epithelium with numerous infoldings associated with mitochondria.

Figure 23. Epipodite, adult (×14000). Detail of association between mitochondriae and infoldings.

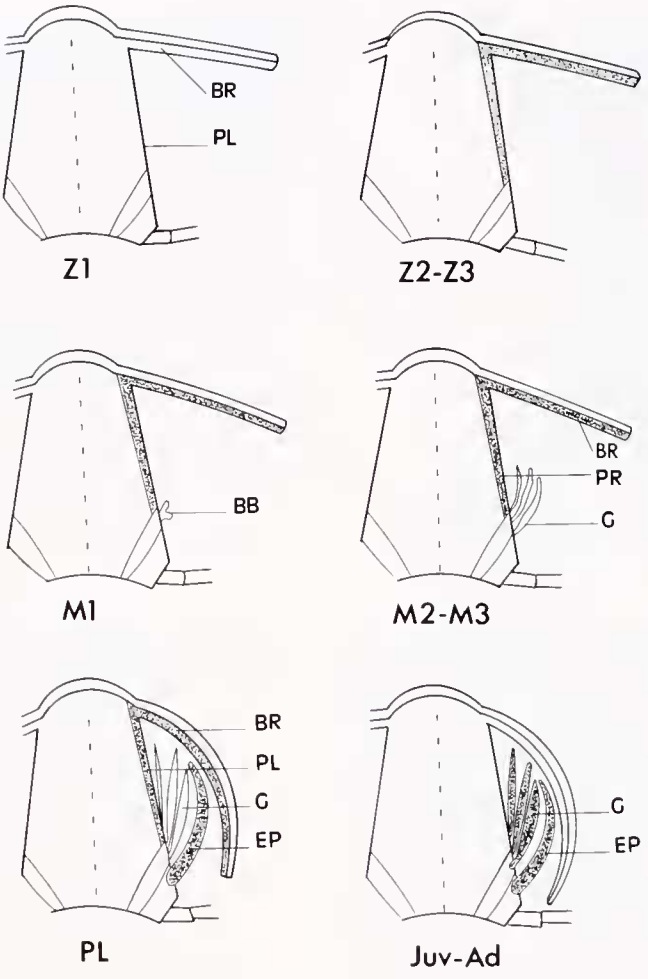


Figure 24. Ontogeny of osmoregulatory epithelia (dotted areas) throughout the postembryonic development of *Penaeus japonicus*; schematic transverse sections through the cephalothorax. Z: zoeae, M: mysis, PL: postlarva, Juv-Ad: juvenile and adult, BR: branchiostegite, EP: epipodite, G: gills, PR: pleura.

In young larvae (nauplii and zoea 1), osmoregulatory structures that can be related to the nonexistent ATPase activity are absent (Bouaricha *et al.*, 1991), because Na^+-K^+ ATPase is located in basolateral membranes of ion-transporting tissues (Towle, 1981; Towle, 1984a, b; Towle and Kays, 1986). These stages are probably osmoconformers and are not tolerant to low salinities (Charmantier *et al.*, 1988).

In later larval stages (mysis 2–3), osmoregulatory tissues are present in the branchial chambers, on the inner side of the branchiostegites, and on the pleurae. The gills and the first epipodites appear as buds at these stages (Hudinaga, 1942; Bouaricha, 1990). The activity of Na^+-K^+ ATPase increases slightly, but these stages retain an osmoconforming type of regulation and a low tolerance to variations in salinity.

After metamorphosis, *i.e.*, in postlarvae, osmoregulatory tissues disappear from the branchiostegites and pleurae while appearing during the postlarval phase in the gills and mainly in the epipodites. ATPase activity increases correlatively and reaches a maximum in PL5 and, to a lesser extent, in PL 6 (Bouaricha *et al.*, 1991). Osmotic regulation becomes hyper- and hypoosmotic starting in PL 1, and hyper- and hypoosmoregulatory capacities reach a maximum in PL 5–6 and later stages (Charmantier *et al.*, 1988). Salinity tolerance also increases in PL 5–6 and later stages; under natural conditions, these stages will have migrated from the open sea to coastal, lagunal, and estuarine habitats that are subject to ample fluctuations in salinity.

In conclusion, the ontogeny of osmoregulatory structures described in this study is strongly correlated with the ontogeny of the physiological processes of osmoregulation, and both are correlated with the ecology of *P. japonicus*.

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